

## CLAIMS:

1. A nucleic acid encoding the carboxy-terminal portion of the heavy chain ( $H_C$ ) of botulinum neurotoxin (BoNT) selected from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype C<sub>1</sub>, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G, wherein said nucleic acid is expressable in a recombinant organism selected from *Escherichia coli* and *Pichia pastoris*.
2. The nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid sequence selected from SEQ ID No:1 (serotype A), SEQ ID No:7 (serotype B), SEQ ID No:9 (serotype C<sub>1</sub>), SEQ ID No:11 (serotype D), SEQ ID No:13 (serotype E), SEQ ID No:15 (serotype F), and SEQ ID No:17 (serotype G).
3. The nucleic acid of claim 1, wherein the nucleic acid encodes an  $H_C$  amino acid sequence of BoNT selected from SEQ ID No:2 (serotype A), SEQ ID No:8 (serotype B), SEQ ID No:10 (serotype C<sub>1</sub>), SEQ ID No:12 (serotype D), SEQ ID No:14 (serotype E), SEQ ID No:16 (serotype F), and SEQ ID No:18 (serotype G).
4. A nucleic acid encoding the amino-terminal portion of the heavy chain ( $H_N$ ) of botulinum neurotoxin (BoNT) selected from the group consisting of BoNT serotype B, BoNT serotype C<sub>1</sub>, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G, wherein said nucleic acid is expressable in a recombinant organism selected from *Escherichia coli* and *Pichia pastoris*.
5. The nucleic acid of claim 4, wherein said nucleic acid comprises a nucleic acid sequence selected from SEQ ID No:21 (serotype B), SEQ ID No:23 (serotype C<sub>1</sub>), SEQ ID No:25 (serotype D), SEQ ID No:27 (serotype E), SEQ ID No:29 (serotype F), and SEQ ID No:31 (serotype G).
6. The nucleic acid of claim 4, wherein the nucleic acid encodes an  $H_N$  amino acid sequence of BoNT selected from SEQ ID No:22 (serotype B), SEQ ID No:24 (serotype C<sub>1</sub>), SEQ ID No:26 (serotype D), SEQ ID No:28 (serotype E), SEQ ID No:30 (serotype F), and SEQ ID No:32 (serotype G).

7. The nucleic acid of any one of claims 1, 3, 4, or 6, wherein the sequence of the nucleic acid is designed by selecting at least a portion of the codons encoding H<sub>C</sub> from codons preferred for expression in a host organism.

8. The nucleic acid of claim 7, wherein the host organism is selected from gram negative bacteria, yeast, and mammalian cell lines.

9. The nucleic acid of claim 8, wherein the host organism is *Escherichia coli* or *Pichia pastoris*.

10. The nucleic acid of any one of claims 1, 3, 4, or 6, wherein the nucleic acid sequence encoding H<sub>C</sub> is designed by selecting codons encoding H<sub>C</sub> which codons provide H<sub>C</sub> sequence enriched in guanosine and cytosine residues.

11. The nucleic acid of any one of claims 1, 3, 4, or 6, wherein said nucleic acid is a synthetic nucleic acid.

12. The nucleic acid of any one of claims 1, 3, 4, or 6, wherein said nucleic acid encoding H<sub>C</sub> or H<sub>N</sub> is expressed in a recombinant host organism with higher yield than a second nucleic acid fragment encoding the same H<sub>C</sub> sequence, said second nucleic acid fragment having the wild-type *Clostridium botulinum* sequence of H<sub>C</sub>.

13. An expression vector comprising the nucleic acid of any one of claims 1, 3, 4, or 6, whereby H<sub>C</sub> or H<sub>N</sub> is expressed upon transfection of a host organism with said expression vector.

14. A method of preparing a polypeptide comprising the carboxy-terminal portion of the heavy chain (H<sub>C</sub>) of botulinum neurotoxin (BoNT) or the amino-terminal portion of the heavy chain (H<sub>N</sub>) of botulinum neurotoxin (BoNT) selected from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype C, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G,

said method comprising culturing a recombinant host organism transfected with the expression vector of claim 13 under conditions wherein  $H_C$  or  $H_N$  is expressed.

15. The method of claim 14, wherein the recombinant host organism is a eukaryote.
16. The method of claim 14, further comprising recovering insoluble protein from said host organism, whereby a fraction enriched in H<sub>C</sub> or H<sub>N</sub> is obtained.
17. The method of claim 16, wherein said host organism is *Pichia pastoris*.
18. An immunogenic composition comprising the carboxy-terminal portion of the heavy chain (H<sub>C</sub>) of botulinum neurotoxin (BoNT) selected from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype C, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G.
19. The immunogenic composition of claim 18, wherein H<sub>C</sub> is prepared by culturing a recombinant organism transfected with an expression vector encoding H<sub>C</sub>.
20. The immunogenic composition of claim 19, wherein an insoluble protein fraction enriched in H<sub>C</sub> is recovered from said recombinant organism.
21. An immunogenic composition comprising the amino-terminal portion of the heavy chain (H<sub>N</sub>) of botulinum neurotoxin (BoNT) selected from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype C, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G.
22. The immunogenic composition of claim 21, wherein H<sub>N</sub> is prepared by culturing a recombinant organism transfected with an expression vector encoding H<sub>N</sub>.

23. The immunogenic composition of claim 22, wherein an insoluble protein fraction enriched in H<sub>N</sub> is recovered from said recombinant organism.

24. An immunogenic composition comprising a polypeptide comprising epitopes  
5 contained in the carboxy-terminal portion of the heavy chain (H<sub>C</sub>) of botulinum neurotoxin (BoNT) or the amino-terminal portion of the heavy chain (H<sub>N</sub>) of botulinum neurotoxin (BoNT) selected from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype C, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G, said epitopes eliciting protective immunity  
10 toward the respective BoNT serotype.

25. The immunogenic composition of claim 24, wherein said immunogenic composition elicits an ELISA response to the respective BoNT serotype in an animal, said ELISA response being detectable upon 100-fold dilution of serum from  
15 said animal.

26. An immunogenic composition comprising a protein containing at least a portion of a botulinum neurotoxin (BoNT) sequence, said BoNT being selected from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype C,  
20 BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G.

27. The immunogenic composition of claim 26, wherein said portion of BoNT sequence elicits an ELISA response to the respective BoNT serotype in an animal, said ELISA response being detectable upon 100-fold dilution of serum from said  
25 animal.

28. The immunogenic composition of claim 26, wherein said protein is a fusion protein further comprising a non-toxic polypeptide sequence.

29. The immunogenic composition of claim 26, wherein said composition is endotoxin free.

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30. The immunogenic composition of claim 26, wherein the portion of BoNT sequence is a portion of carboxy-terminal heavy chain (H<sub>C</sub>) sequence or amino-terminal heavy chain (H<sub>N</sub>) sequence.
- 5 31. A nucleic acid encoding a protein containing at least a portion of a botulinum neurotoxin (BoNT) sequence, said BoNT being selected from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype C, BoNT serotype D, BoNT serotype E, BoNT serotype F, and BoNT serotype G.
- 10 32. The nucleic acid of claim 31, wherein said protein containing at least a portion of BoNT sequence elicits an ELISA response to the respective BoNT serotype in an animal, said ELISA response being detectable upon 100-fold dilution of serum from said animal.
- 15 33. An expression vector comprising the nucleic acid of claim 31.
34. A recombinant host cell containing the expression vector of claim 33.
- 35 The recombinant host cell of claim 34, wherein said host cell expresses a protein containing at least a portion of BoNT sequence, said portion of BoNT sequence containing at least one protective epitope of the respective BoNT serotype.
- 20 36. The recombinant host cell of claim 34, wherein said host cell expresses a protein containing at least a portion of BoNT sequence, and wherein said protein elicits an ELISA response to the respective BoNT serotype in an animal, said ELISA response being detectable upon 100-fold dilution of serum from said animal.
- 25 37. The recombinant host cell of claim 34, wherein said host cell expresses a protein containing at least a portion of BoNT sequence, said protein making up at least 0.75% of the total cellular protein.
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38. The recombinant host cell of claim 34, wherein said host cell expresses a protein containing at least a portion of BoNT sequence, said protein making up at least 20% of the total cellular protein.

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